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# Corresponding-site interference, synaptinemal complex structure, and 8+:0*m* and 7+:1*m* octads from *wild-type* × *mutant* crosses of *Ascobolus immersus*

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SUMMARY

'Wider ratio' octads (8:0, 0:8, 7:1 and 1:7) regularly occurred in *wild-type*(+) × *white* ascospore(*w*) crosses of the Pasadena strains of *Ascobolus*. Control crosses showed that phenocopies and false octad clusters were absent or rare; no reversion from *w* to + occurred, but mutation from + to *w* was found at a number of loci, with nearly all 0+:8*w* and many 2+:6*w* octads in + × *w* crosses arising from mutation, not conversion. Nearly all 8+:0*w*, 7+:1*w* and 6+:2*w* octads appeared to arise by conversion.

The finding of genuine wider ratio octads implies hybrid-DNA formation at corresponding sites in *both* pairs of non-sister chromatids in the same bivalent, which conflicts with models of the synaptinemal complex requiring that only two of the four chromatids pair intimately at any point. Octad types arising from hybrid-DNA formation at corresponding sites in both pairs of non-sister chromatids were described and formulae were derived for their frequencies. The lack of genuine wider ratio octads in several other *Ascobolus* studies was shown to be explicable quantitatively in terms of their conversion frequencies.

'Corresponding-site interference' is defined as interference between the two pairs of non-sister chromatids of a bivalent in hybrid-DNA formation at exactly corresponding sites. Formulae based on observed octad frequencies were derived for calculating coincidence values for this kind of interference. Corresponding-site interference was found to be weak, with coincidence values differing between crosses with high and with low conversion frequencies.

## 1. INTRODUCTION AND DERIVATION OF FORMULAE

*Wild-type*(+) × *mutant*(*m*) crosses typically give 1:1 segregation ratios in the products of meiosis: 2+:2*m* in tetrads, and 4+:4*m* where a further mitosis gives an octad of 8 spores, with sister spores (those from the same mitosis) being identical. Aberrant ratios such as 6+:2*m*, 2+:6*m*, 5+:3*m* and 3+:5*m* were, however, reported for ascospore colour markers in fungi (Olive, 1959; Lissouba, Mousseau, Rizet & Rossignol, 1962, and others). Postmeiotic segregation, resulting in non-identical sister-spores, is shown in 5:3 and 3:5 octads (one pair of non-identical

spores), and Kitani, Olive & El-Ani (1962) discovered aberrant 4:4 octads with two pairs of non-identical sister spores. 6:2, 2:6, 5:3 and 3:5 octads (here called 'narrower ratios') and aberrant 4:4s have been explained in terms of hybrid-DNA formation between one pair of non-sister chromatids of a bivalent, with subsequent correction or non-correction of mis-paired bases at a site of allele difference in the cross (Whitehouse, 1963; Holliday, 1964; Emerson, 1966).

The present work investigated the possibility of hybrid-DNA formation at corresponding sites in *both* pairs of non-sister chromatids of a single bivalent. Whether there is interference between the two pairs of non-sister chromatids in hybrid-DNA formation at corresponding sites is relevant to conversion and also to synaptinemal complex structure. This kind of interference will be called 'corresponding-site interference' and is quite distinct from classical chromosome (crossover position) and chromatid (strand) interference which refer to successive crossovers along a chromosome, not to single sites in corresponding positions in the two pairs of non-sister chromatids. At least one model (von Wettstein, 1971) of the synaptinemal complex implies complete positive corresponding-site interference, with only two of the four chromatids intimately paired at any point.

Hybrid-DNA formation involving the same site in both pairs of non-sister chromatids in a single bivalent could give rise to 'wider ratio' aberrant ratios, $8+:0m$, $0+:8m$, $7+:1m$ and $1+:7m$, and to 6:2, 5:3 and 4:4 octads with 2, 3 and 4 pairs, respectively, of non-identical sister spores (Table 1). If conversion occurs according to the original kind of hybrid-DNA model (Whitehouse, 1963; Holliday, 1964) with hybrid-DNA forming symmetrically in two non-sister chromatids, wider ratio octads would arise from hybrid-DNA formation at corresponding sites in all four chromatids. If the single hybrid chromatid hypotheses (Whitehouse, 1967; Paszewski, 1970; Stadler & Towe, 1971) are correct, all four chromatids would be involved in hybrid-DNA formation, but it would only form at that site in one of each pair of non-sister chromatids in the bivalent.

The two pairs of non-sister chromatids in a bivalent should be genetically and biochemically identical: for example, if sister pairs from replication of homologous parental chromosomes A and B are called A,A and B,B, there are two non-sister pairs, each A,B. If hybrid-DNA formation and the correction of mis-pairing are independent in the two pairs, the frequency of combinations of events in the two pairs will be the product of the frequencies of individual events in each non-sister pair. The events per pair of non-sister chromatids, their frequencies and their consequences in the case of hybrid-DNA forming at a site in only one such pair are:

$4+:0m$, frequency $a$ giving a $6+:2m$ octad; $0+:4m$, $b$, $2+:6m$; $3+:1m$, $c$, $5+:3m$; $1+:3m$, $d$, $3+:5m$; $2+:2m$, with two pairs of non-identical sister spores, $e$, aberrant 4:4; $2+:2m$ with identical sister spores. $f$, normal 4:4.

Table 1 shows the consequences of combinations of these events if hybrid-DNA forms at corresponding sites in both pairs of non-sister chromatids. The expected

octad frequencies are as follows, with the number of non-identical sister spore pairs given in brackets:

(i) Unique classes only obtained from hybrid-DNA formation in *both* pairs of chromatids:

$8+:0m,\ a^2;\quad 0+:8m,\ b^2;\quad 7+:1m\ (1),\ 2ac;\quad 1+:7m\ (1),\ 2bd;\ 6+:2m\ (2),$
$2ae+c^2;\quad 2+:6m\ (2),\ 2be+d^2;\quad 5+:3m\ (3),\ 2ce;\quad 3+:5m\ (3),\ 2de;$
$4+:4m\ (4),\ e^2.$

(ii) Non-unique classes, obtained from hybrid-DNA formation in both or in only one pair of chromatids:

$6+:2m,\ 2af;\quad 2+:6m,\ 2bf;\quad 5+:3m\ (1),\ 2ad+2cf;\quad 3+:5m\ (1),$
$2bc+2df;\quad 4+:4m,\ 2ab+f^2;\quad 4+:4m\ (3),\ 2cd+2ef.$

Because the unique classes are usually absent – or are very rare when detectable – the occurrence of non-unique classes as a result of hybrid-DNA formation in both

Table 1. *Expected octad types after hybrid-DNA formation at corresponding sites in both pairs of non-sister chromatids of a bivalent*

| | | Ratio in one pair of non-sister chromatids | | | | | |
|---|---|---|---|---|---|---|---|
| | | 4+:0w | 3+:1w (1)* | 1+:3w (1) | 0+:4w | 2+:2w (2) | 2+:2w |
| Ratio in the other pair | 4+:0w | 8+:0w | 7+:1w (1) | 5+:3w (1) | 4+:4w | 6+:2w (2) | 6+:2w |
| | 3+:1w (1)* | 7+:1w (1) | 6+:2w (2) | 4+:4w (2) | 3+:5w (1) | 5+:3w (3) | 5+:3w (1) |
| | 1+:3w (1) | 5+:3w (1) | 4+:4w (2) | 2+:6w (2) | 1+:7w (1) | 3+:5w (3) | 3+:5w (1) |
| | 0+:4w | 4+:4w | 3+:5w (1) | 1+:7w (1) | 0+:8w | 2+:6w (2) | 2+:6w |
| | 2+:2w (2) | 6+:2w (2) | 5+:3w (3) | 3+:5w (3) | 2+:6w (2) | 4+:4w (4) | 4+:4w (2) |
| | 2+:2w | 6+:2w | 5+:3w (1) | 3+:5w (1) | 2+:6w | 4+:4w (2) | 4+:4w |

* Figure in parentheses is the number of non-identical sister spore pairs.

pairs of non-sister chromatids should also be extremely rare, so the frequencies of $4+:0m$, $0+:4m$, $3+:1m$ (1), $1+:3m$ (1) and $2+:2m$ (2) segregations in a single pair of non-sister chromatids will approximate very closely to half the frequencies of $6+:2m$, $2+:6m$, $5+:3m$ (1), $3+:5m$ (1) and $4+:4m$ (2) octads, respectively, giving values for $a$, $b$, $c$, $d$ and $e$. The halving is necessary because a narrower ratio octad, for example $6+:2m$, results from a conversion event, in this example $4+:0m$, in one of two possible pairs of non-sister chromatids.

It is therefore possible to determine qualitatively whether hybrid-DNA occurs at corresponding sites in both pairs of non-sister chromatids by the presence or absence of the unique classes listed above, and to determine quantitatively whether

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hybrid-DNA occurs independently in both pairs of chromatids by comparing the observed frequencies of the unique classes with expected frequencies calculated from the formulae above using values of *a*, *b*, *c*, *d* (and *e* if aberrant 4:4s can be detected) obtained from the same data.

In previous studies, Kitani *et al.* (1962) reported one 7+ : 1*gray* octad in a sample of about 200000 in *Sordaria fimicola*, but this is too rare for useful quantitative studies. 8:0 and 7:1 octads were observed from the European strains of *Ascobolus immersus* (e.g. by Kruszewska & Gajewski, 1967; Paszewski & Prazmo, 1969; Rossignol, 1969; Baranowska, 1970), but they found these to be spurious. They found spontaneous mutation from + to *white* at a number of loci, giving 0+ : 8*w*s; phenocopies, where octads with wider ratio phenotypes were genotypically 4:4; and false clusters, where in the shot octads scored, less than eight spores from each of two or more asci landed together on the collecting surface, giving chance clusters of eight spores, mostly with non-4:4 mating-type ratios. Emerson (1969) reported rare occurrences of 8+ : 0*w*s in the American strains of *Ascobolus immersus*, but suggested that asci in which more than six members of the octads carry one allele are probably sufficiently infrequent to be accounted for by some occurrence which is independent of conversion, such as mutation.

The present study used the American (Pasadena) strains of *Ascobolus immersus*, kindly provided by Professor Sterling Emerson. These often have very high conversion frequencies, with aberrant ratios exceeding 10% in some crosses (Emerson & Yu-Sun, 1967) so that the expected frequencies of rare conversion classes are much higher than in previously reported *Sordaria* and *Ascobolus* crosses. Some of the present findings were summarized in a previous abstract (Lamb, 1972).

## 2. MATERIALS AND METHODS

Strains, media and methods were as described by Emerson & Yu-Sun (1967). Their paper describes the origin of high conversion frequency strains *w*-10(P) and *w*-78(P) from the original mutant strains *w*-10 and *w*-78 (here called low conversion strains). *w*-10 and *w*-78 both result in white ascospores and are closely linked, possibly allelic, but are not linked to another white mutation, *w*-62.

The main experiments consisted of different + × *w* crosses, made at 10, 17·5, or 22·5 °C, and scoring shot octads collected on 1½% water agar in lids inverted over the crosses. The crosses were given continuous illumination by six 8 W fluorescent tubes.

As controls, + × + crosses were made to test for spontaneous mutation from + to *white*, and *w*-78 × *w*-78 and *w*-10 × *w*-10 crosses were made to test for reversion from *white* to + (browny red). From + × *w* crosses, hundreds of octads with aberrant ratios were isolated for germination and backcrossing to appropriate parental strains. This was a check for phenocopies and also for false clusters as the latter frequently give non-4:4 mating-type ratios.

In the main experiments and controls, octads with pale pink spores were ignored, as were spore clusters with more or fewer than eight spores.

## 3. RESULTS

### (i) *Controls*

There was no reversion to + in any $w \times w$ crosses. Crosses between various high and low conversion strains were made at 10 and 17·5 °C, but no red(+) spores were observed in 26249 octads from $w$-10 $\times$ $w$-10 crosses, and none in 78591 octads from $w$-78 $\times$ $w$-78 crosses.

Forward mutation from + to *white* was, however, observed in + × + crosses, and by crossing isolates from such white spores to $w$-10 and $w$-78 strains, it was shown that mutations were usually at *white* loci other than, and unlinked to, $w$-10 and $w$-78: none tested were allelic with $w$-10 or $w$-78. In a given + × + cross, the frequency of 4:4 octads from spontaneous mutation often varied in repeat experiments: in one cross, from 1·2(± 0·2)% to 0·02(± 0·02)%; in another, from 0·4(± 0·1)% to 0·02(± 0·01)%; less extreme variation also occurred. The source of variation was easily traced: these 4:4 octads were often clustered on collecting lids and in corresponding positions on successive lids. By direct observation of apothecia, it was often seen that one apothecium or an area of apothecia gave rise to 4:4 octads, showing that the mutation had occurred once in the hyphae, not independently in many asci, so that the frequency of 4:4 octads depended on how early the mutation occurred in a cross's development, as well as on mutation frequencies.

In tests for phenocopies and false clusters, over 300 octads with aberrant phenotypic ratios were isolated from + × $w$ crosses and the spores were germinated for backcrossing to determine genotypes. All red spores and definitely pink spores proved to be genotypically + and all white spores were genotypically $w$ except for two 0+ : 8$w$ octads which were genotypically 4+ : 4$w$. Phenocopies were thus extremely rare and only involved octads with a majority of white spores. Some white spores from 2+ : 6$w$ and 0+ : 8$w$ octads gave 4+ : 4$w$, 2+ : 6$w$ and 0+ : 8$w$ octads when backcrossed to $w$-78 or $w$-10, showing that some 2+ : 6$w$ and most 0+ : 8$w$ octads from the + × $w$ crosses arose by new spontaneous mutations (usually unlinked to $w$-78 or $w$-10), not by conversion. A clustering of mutation-produced 2+ : 6$w$ and 0+ : 8$w$ octads was sometimes noticed and direct observation of apothecia confirmed that one mutation during cross development could give rise to several or many asci showing that mutation. Plate 1 shows an area of a + × $w$-78(P) cross where mutation in a hypha from + to $w$ at a locus closely linked to $w$-78 gave rise to an area of apothecia with 0+ : 8$w$ asci predominating. Large differences in 2+ : 6$w$ frequencies between repeat + × $w$ crosses occasionally occurred – for example, 13·2(± 0·06)% and 2·2(± 0·2)%. In such an example, the lower value is usually a better estimate of conversion than the higher one, which is often raised by early occurrence of a $w$ mutation in the cross. Similar large differences were not found for 6+ : 2$w$ frequencies between repeat crosses, as expected in the absence of reversion.

In these backcrossing experiments with over 300 aberrant ratio octads, only one presumed false cluster was found. This was an 8+ : 0$w$ octad from a low

conversion $+ \times w$-10 cross with $0(+):8(-)$ for mating type. As false clusters were so rare, they probably resulted only in very small increases in the observed aberrant ratio frequencies. Two other experiments, as well as indirect evidence, showed that false clusters were not responsible for any substantial proportion of wider ratio octads scored. In *Ascobolus*, the mature, undehisced ascus has its base below the apothecial surface (Plate 1, fig. 2) but with a needle the ascus can often be eased out intact (Plate 1, fig. 3) for scoring or testing. Such isolated asci were scored from three crosses on a number of days and these scores were compared with those from octads shot from the same crosses onto collecting lids at about the same period. There was excellent agreement between the two sets of aberrant ratio frequencies except that $0+:8ws$ were much more frequent in intact asci than in shot octads, suggesting that some intact $0+:8ws$ were immature, with genotypically $+$ spores not yet pigmented. Pigmentation must generally precede dehiscence, as only two of many $0+:8ws$ tested contained genotypically $+$ spores.

Intact asci with phenotypic ratios $8+:0w$, $6+:2w$ and $5+:3w$ from $+ \times w$ crosses were tested by germinating spores and backcrossing: all genotypes corresponded completely with phenotypes and all had $4(+):4(-)$ mating-type ratios. This proved that genuine wider ratio octads occur. Crosses involving reisolates from wider ratio octads showed no regular spore-abortion patterns or other signs of chromosomal rearrangements or aneuploidy.

In comparison, Stadler, Towe & Rossignol (1970) tested octads with aberrant ratios for other $w$ loci in the Pasadena strains of *Ascobolus*. They found that nearly all $6+:2ws$ were valid; $5+:3ws$, $3+:5ws$ and $2+:6ws$ were all or mostly genuine for some alleles but all or mostly spurious for others. Some $2+:6ws$ and all but one $0+:8w$ octads arose by mutation: no $8+:0w$ or $7+:1ws$ were reported.

In summary, the present controls showed: that genuine wider ratio $8+:0w$ and $7+:1w$ octads occur; that reversion, phenocopies and false clusters were absent or extremely rare. Because of mutation to $w$, frequencies of conversion to $w$ will be overestimated in scores of octad phenotypes from $+ \times w$ crosses. Frequencies of conversion to $+$ are probably not appreciably biased, so in the main results analysis will deal largely with $8+:0w$, $7+:1w$, $6+:2w$ and $5+:3w$ octads, especially $8+:0w$ and $6+:2ws$ as octads of these classes were most fully tested by back crossing.

### (ii) *Main experiments*

Wider ratio octads $8+:0w$, $0+:8w$, $7+:1w$ and $1+:7w$ were found in most crosses: their frequencies in different crosses of $+$ with $w$-78 and $w$-10 are given in Table 2. Plate 2, figs. 4–9, show some of the octad types scored. Some wider ratio types were not found in all crosses, especially where conversion frequencies were low and sample sizes small, but would probably occur in larger samples. The four wider ratio octad types were found in $+ \times w$-62 crosses, but samples were small and controls much less extensive than for $w$-10 and $w$-78.

Replicate and repeat experiments usually gave homogeneous results for conversion classes with more $+$ spores than $w$. Results in Table 2 are from pooled

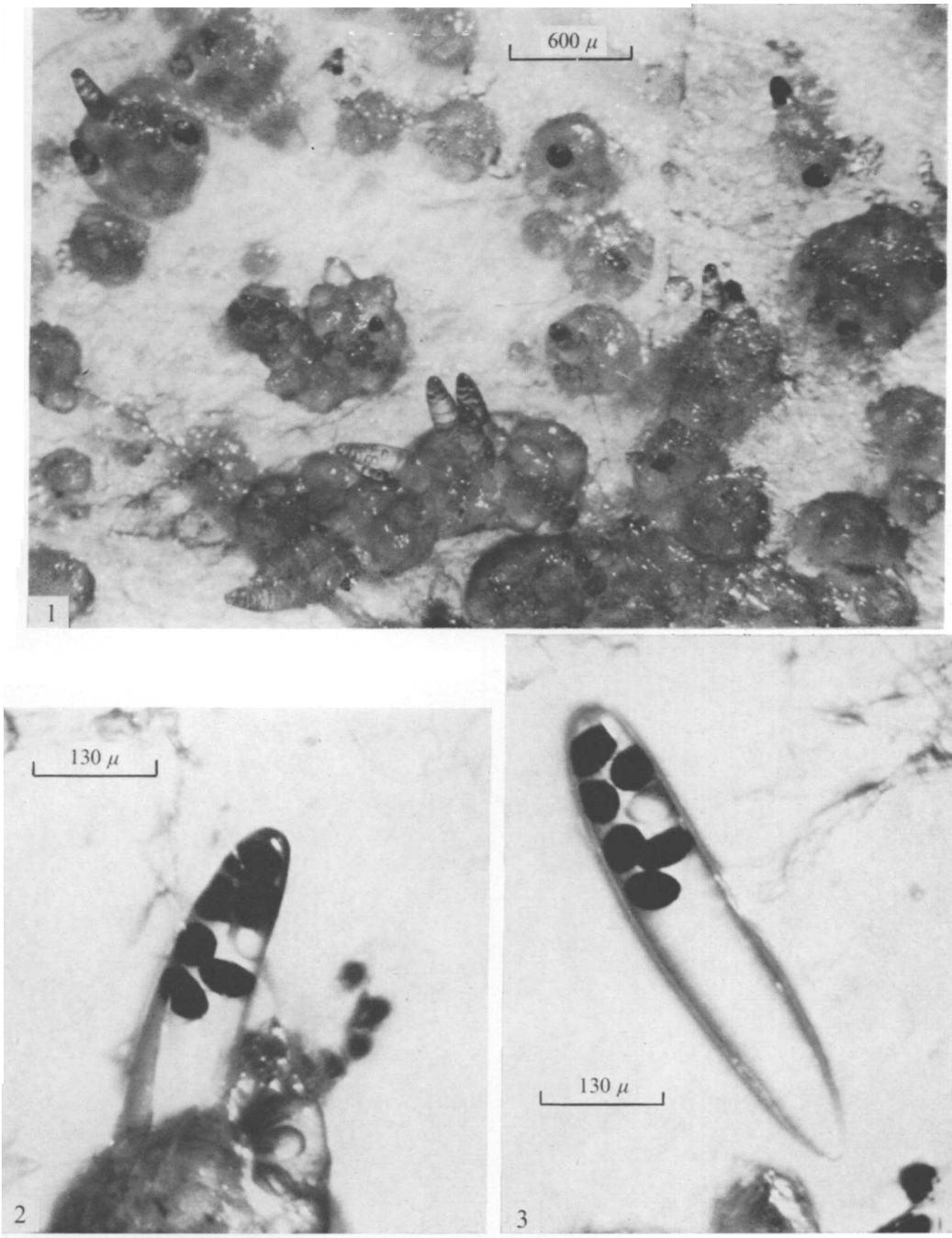


Fig. 1. Apothecia with intact asci from a + × *w*-78(P) cross. A single mutation from + to *w* at a locus fairly close to *w*-78 occurred early in the cross's development, giving rise to 0+ : 8*w* asci in several apothecia. Most of the other asci visible, with red spores as well as white, do not carry the new mutation.

Fig. 2. Intact, undehisced 6+ : 2*w* ascus (one *w* spore hidden) with its base below the apothecial surface. + × *w*-10(P).

Fig. 3. The 6+ : 2*w* ascus shown in Fig. 2, after being isolated intact by easing the ascus base clear of apothecium. + × *w*-10(P).



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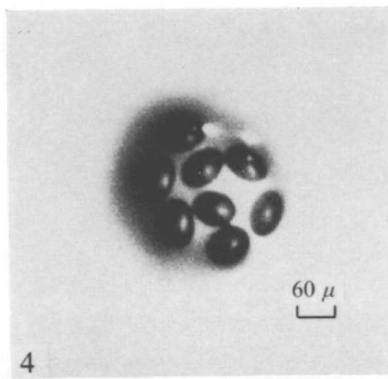


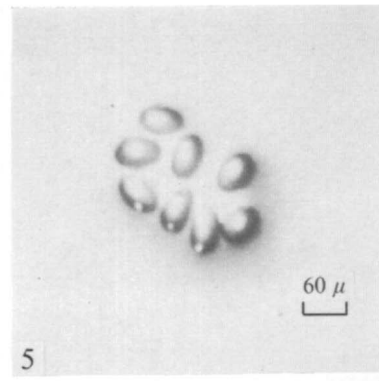


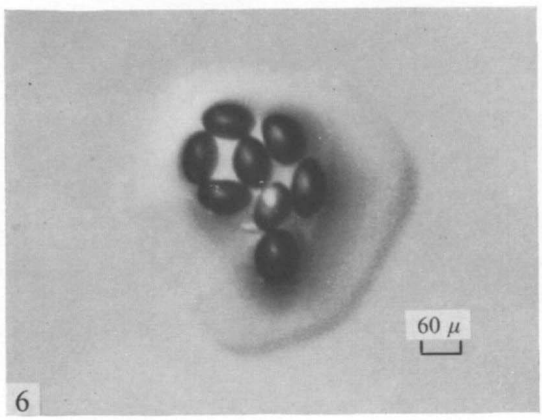


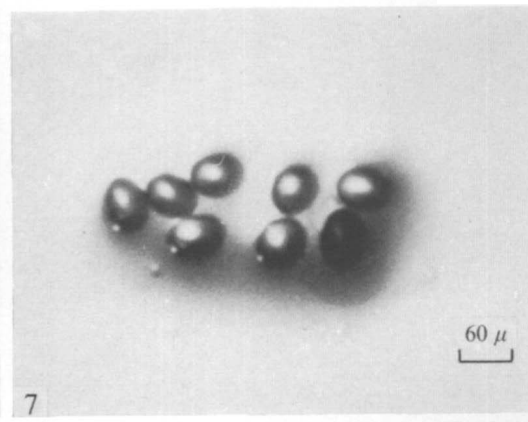


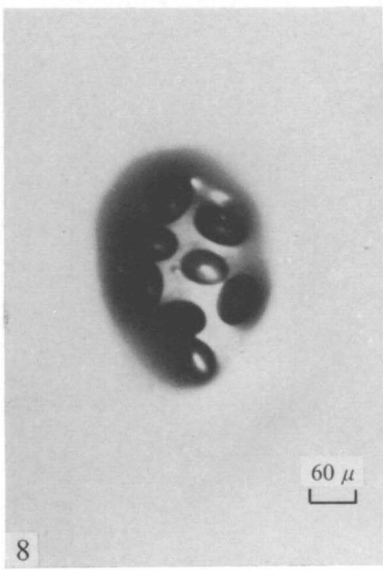


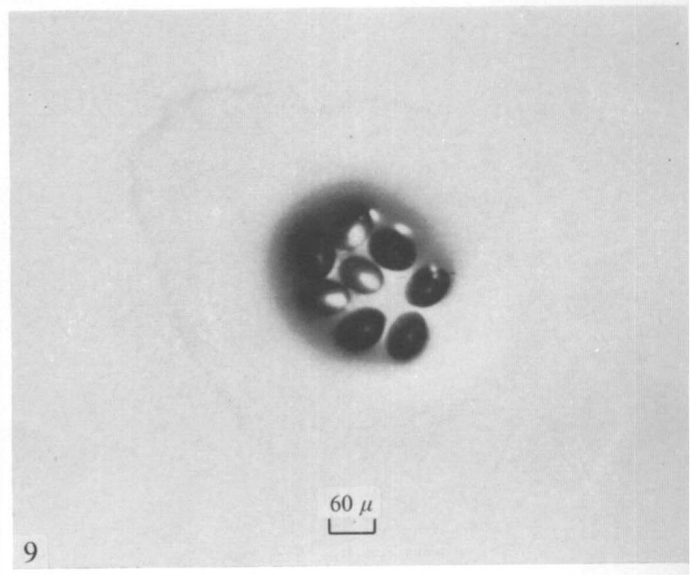


Figs. 4–9. Shot octads on collecting agar surfaces. In some, the shot ascal sap, sometimes containing small refractive globules, is visible around the octads.

Fig. 4. 8+:0*w*. + × *w*-10(P) reisolate.

Fig. 5. 0+:8*w*. + × *w*-10(P).

Fig. 6. 7+:1*w*. + × *w*-10(P).

Fig. 7. 1+:7*w*. + × *w*-10(P).

Fig. 8. 6+:2*w*. + × *w*-78(P).

Fig. 9. 5+:3*w*. + × *w*-10.



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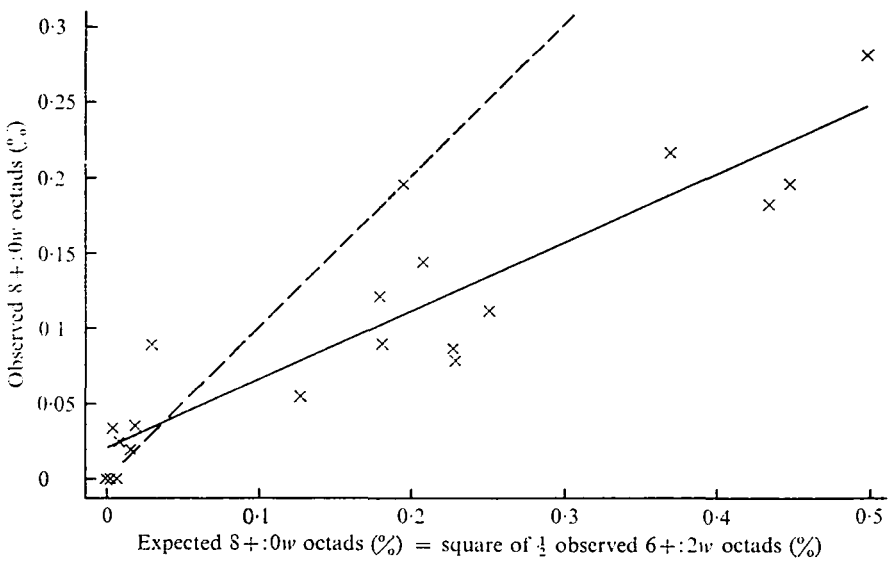
data from crosses giving similar, usually homogeneous (at the $P = 0{\cdot}05$ level) results. Many individual samples had no or extremely few octads in some of the rarer classes, so full homogeneity testing was not done for each octad class.

Table 2. *Octad frequencies in different crosses*

| Temp. (°C) | Conversion range | Octad ratios, +:*w* (%) 4:4 | 6:2 | 2:6 | 5:3 | 3:5 | 7:1 | 1:7 | 8:0 | 0:8 | Total octads |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | + × *w*-78 | | | | | | |
| 10·0 | Low | 97·94 | 0·26 | 0·73 | 0·31 | 0·09 | 0·00 | 0·01 | 0·00 | 0·66 | 23 503 |
| 10·0 | High | 94·13 | 2·86 | 1·98 | 0·47 | 0·31 | 0·00 | 0·00 | 0·01 | 0·23 | 9 845 |
| 17·5 | Low | 96·05 | 1·49 | 1·80 | 0·23 | 0·19 | 0·005 | 0·01 | 0·005 | 0·22 | 21 563 |
| 17·5 | High | 80·63 | 7·07 | 10·63 | 0·78 | 0·40 | 0·02 | 0·05 | 0·05 | 0·38 | 5 760 |
| | | | | | + × *w*-10 | | | | | | |
| 10·0 | Low | 98·07 | 0·14 | 1·13 | 0·20 | 0·03 | 0·00 | 0·00 | 0·03 | 0·40 | 3 530 |
| 10·0 | High | 94·50 | 3·71 | 1·51 | 0·08 | 0·07 | 0·00 | 0·007 | 0·03 | 1·11 | 15 134 |
| 17·5 | Low | 95·69 | 1·27 | 1·98 | 0·13 | 0·18 | 0·01 | 0·01 | 0·02 | 0·71 | 18 034 |
| 17·5 | High | 85·39 | 10·25 | 3·61 | 0·14 | 0·09 | 0·01 | 0·002 | 0·13 | 0·37 | 80 973 |




Text-fig. 1. Relation between observed $8+:0w$ frequencies and expected $8+:0w$ frequencies calculated from observed $6+:2w$ frequencies in various $+ \times w$-10 crosses at 17·5 °C. ——, Calculated regression line, $y = 0{\cdot}021 + 0{\cdot}450x$; – –, Expected line if corresponding-site interference is absent, $y = 0 + 1x$.

The results show that the frequencies of wider ratio and narrower ratio octads varied markedly with the strains used (high or low conversion crosses). The origin of most of the high and low conversion strains used was described by Emerson & Yu-Sun (1967), but in the present work new strains isolated from crosses were also used, giving intermediate as well as high and low conversion crosses. The spread

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of conversion frequencies obtained for a single mutation ($w$-10) at a single temperature (17·5 °C) is apparent from Text-fig. 1 for the $6+:2w$ and $8+:0w$ classes.

As outlined in the introduction, $8+:0w$ frequencies are expected to be related to the square of half the $6+:2w$ frequencies. While there was an overall relationship of this kind in all the data, it shows most clearly in results for a single mutation at a single temperature, as in Text-fig. 1 which shows the most extensive data of this kind. There is a clear positive correlation of $8+:0w$ frequencies with the square of half the $6+:2w$ frequencies. The calculated regression, $y = 0{\cdot}0206 + 0{\cdot}450x$, has a slope significantly different from 0 and from 1, at the $P = 0{\cdot}001$ level.

Temperature affected the frequency of narrower and wider ratio octads (Table 2). The frequency of $6+:2w$ octads increased several-fold with a rise from 10 to 17·5 °C in $w$-10 and $w$-78 high and low conversion crosses: these increases were all significant at $P = 0{\cdot}01$. The $8+:0w$s showed a significant rise over this temperature interval for $w$-10 high conversion crosses, but for other crosses samples were too small for significant trends to be established. The details of the temperature effects will be considered elsewhere (Lamb & Wickramaratne, unpublished).

### (iii) *Interference between non-sister chromatids in hybrid-DNA formation at corresponding sites*

The frequencies of wider ratio octad classes expected in the absence of corresponding-site interference can be calculated from observed narrower ratio class

Table 3. *Calculation of expected wider ratio frequencies from observed octad class frequencies*

| Wider ratio class* | Formula based on observed octad frequencies* |
|---|---|
| | (i) Simpler formulae† |
| $8+:0w$ | $(\frac{1}{2}6+:2w)^2$ |
| $0+:8w$ | $(\frac{1}{2}2+:6w)^2$ |
| $7+:1w$ | $2(\frac{1}{2}6+:2w \times \frac{1}{2}5+:3w)$ |
| $1+:7w$ | $2(\frac{1}{2}2+:6w \times \frac{1}{2}3+:5w)$ |
| | (ii) Partly corrected formulae‡ |
| $8+:0w$ | $(\frac{1}{2}6+:2w + 8+:0w + \frac{1}{2}7+:1w)^2$ |
| $0+:8w$ | $(\frac{1}{2}2+:6w + 0+:8w + \frac{1}{2}1+:7w)^2$ |
| $7+:1w$ | $2(\frac{1}{2}6+:2w + 8+:0w + \frac{1}{2}7+:1w)(\frac{1}{2}5+:3w + \frac{1}{2}7+:1w)$ |
| $1+7w$ | $2(\frac{1}{2}2+:6w + 0+:8w + \frac{1}{2}1+:7w)(\frac{1}{2}3+:5w + \frac{1}{2}1+:7w)$ |

* Expressions such as $8+:0w$ and $6+:2w$ here stand for the frequencies (not %s) of these octad classes.

† These assume that hybrid-DNA formation at corresponding sites in both pairs of non-sister chromatids is negligibly rare.

‡ These correct the calculations for the occurrence of hybrid-DNA at corresponding sites in both pairs of non-sister chromatids only when this leads to the formation of a wider ratio octad.

frequencies, as described earlier. The formulae are given in Table 3 (i). Comparisons of observed and expected numbers of $8+:0w$ and $7+:1w$ octads are shown in Table 4, and Text-fig. 1 shows more detailed data for various $+ \times w$-10 crosses

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at 17·5 °C. It is clear that neither complete positive, nor complete negative, corresponding-site interference occurred.

Different degrees of interference, related to the conversion frequency of the cross, occurred in different crosses. To simplify data presentation, crosses were allocated to two categories, high or low conversion, according to whether the $6+:2w$ frequency at 17·5 °C was greater or less than 5%. In high conversion

Table 4. *Observed and expected numbers of* $8+:0w$ *and* $7+:1w$ *octads in different crosses*

| Locus | Temp. (°C) | Conversion range | Total octads | Number of $8+:0ws$ Observed | Number of $8+:0ws$ Expected | Number of $7+:1ws$ Oserved | Number of $7+:1ws$ Expected |
|---|---|---|---|---|---|---|---|
| *w*-10 | 10·0 | Low | 26 138 | 1 | 0·3 | 0 | 0·0 |
| | | High | 57 021 | 15 | 24·6 | 1 | 0·5 |
| *w*-78 | 10·0 | Low | 69 106 | 0 | 0·3 | 2 | 0·2 |
| | | High | 27 390 | 4 | 17·5 | 2 | 3·6 |
| *w*-10 | 17·5 | Low | 65 813 | 17 | 6·3 | 6 | 1·3 |
| | | High | 91 579 | 126 | 249·9 | 16 | 6·9 |
| *w*-78 | 17·5 | Low | 94 018 | 14 | 5·3 | 10 | 2·2 |
| | | High | 13 992 | 11 | 25·8 | 5 | 4·8 |
| *w*-10 | 22·5 | Low | 11 328 | 2 | 0·8 | 0 | 0·1 |
| | | High | 17 228 | 37 | 49·7 | 4 | 1·0 |
| *w*-78 | 22·5 | Low | 5 129 | 1 | 0·5 | 2 | 0·1 |
| | | High | 6 733 | 3 | 12·4 | 0 | 2·1 |
| Both loci | All temps. | Low | 271 532 | 35 | 13·5 | 20 | 3·9 |
| | | High | 213 943 | 196 | 379·9 | 28 | 18·9 |
| Grand totals | | | 485 475 | 231 | 393·4 | 48 | 22·8 |

crosses, the expected $8+:0ws$ exceeded the observed ones consistently (and usually significantly at $P = 0{\cdot}05$) for *w*-78 and *w*-10 at all three temperatures used, indicating positive corresponding-site interference with an overall coincidence coefficient of 0·52. In contrast, in the low conversion crosses there was negative interference, overall coincidence coefficient 2·59.

The comparisons of observed and expected $7+:1w$ frequencies are based on rather small samples, but there was a clear tendency for more $7+:1ws$ to occur than expected, especially in low conversion crosses. Negative interference was therefore shown for $7+:1ws$.

The formulae in Table 3(i) are based on the assumption that hybrid-DNA formation at corresponding sites in both pairs of non-sister chromatids is negligibly rare. Because individual wider ratio classes often exceeded 0·1%, the formulae were partly corrected (Table 3, ii) to allow for observed wider ratio octad frequencies. It is not possible to correct directly for cases in which hybrid-DNA in both pairs of non-sister chromatids gives rise to non-unique classes (see Introduction), but further minor corrections could be made where unique classes of 6:2, 5:3 and 4:4 asci with 2, 3 and 4 pairs, respectively, of non-identical sister spores are detectable.

The differences made by the revised formulae are small, though not negligible. For example, in *w*-10 results at 17·5 °C, in low conversion crosses, 17 8+ : 0*w*s were observed compared with 6·3 expected from the simpler formulae and 6·8 from the partly corrected ones; in the high conversion crosses, the corresponding figures were 126 8+ : 0*w*s observed, 249·9 and 264·8 expected; for 7+ : 1*w*s, low conversion, 6 observed, 1·3 and 1·4 expected, and for high conversion crosses, 16 observed, 6·9 and 8·0 expected. Correcting for observed wider ratio octads in the formulae did not change the general conclusions about corresponding-site interference but coincidence values were slightly lower because correction increased the expected frequencies of wider ratio octads.

### (iv) *Calculations based on previous workers' data*

Previous workers (references in Introduction and Table 5) using the European strains of *Ascobolus immersus* observed phenotypes corresponding to wider ratio octad types reported as genuine in the present work with the Pasadena strains,

Table 5. *Expected numbers of wider ratio octads calculated for previous workers' data*

| Authors | Mutation | Total octads | Total aberrant ratios (%) | Expected numbers* 8+ : 0*w* | 0+ : 8*w* | 7+ : 1*w* | 1+ : 7*w* |
|---|---|---|---|---|---|---|---|
| | | *Sordaria fimicola* | | | | | |
| Kitani *et al.* 1962 | *gray* | 130 255 | 0·18 | 0·02 | 0·0003 | 0·04* | 0·001 |
| | | *Ascobolus immersus*, European strains | | | | | |
| Kruszewska & Gajewski, 1967 | 775 | 39 829 | 0·20 | 0·01 | 0·006 | 0 | 0 |
| | 77 | 22 111<br>51 426 | 0·40 | —<br>0·08 | 0·02<br>— | —<br>0 | 0<br>— |
| | 794 | 66 920 | — | 0·03 | — | 0 | — |
| Rossignol, 1969 | 1186 | 23 274 | 0·72 | 0·3 | 0·002 | 0 | 0 |
| | 147 | 10 378 | 5·18 | 1·90 | 1·62 | 0 | 0 |
| Paszewski & Prazmo, 1969 | 84*w* | 28 288 | 1·45 | 0·80 | 0·60 | 0 | 0 |
| Baranowska, 1970 | *w*173 | 145 585 | 0·30 | 0·004 | 0·26 | 0 | 0 |
| | *w*140 | 136 323 | 0·30 | 0·04 | 0·12 | 0 | 0 |

* No genuine wider ratio octads were observed except for one 7+ : 1*gray* in the *Sordaria* data of Kitani *et al.* (1962).

—, Original data not sufficient for calculation.

but on testing, they found their octads of these types were spurious. Although they scored large samples, the conversion frequencies were usually much lower than in the present study, giving very low expected frequencies for wider ratio octads. Table 5 shows results of applying the simpler formulae (Table 3, i) to the larger samples in their *Ascobolus* data, and to the *Sordaria fimicola* data of Kitani *et al.* (1962). Less than two, and usually less than one, of any wider ratio class were expected in all samples considered. Because of the many spurious wider ratio

octads they found, it is not surprising that in the samples of wider ratio octads tested, they found no genuine ones except for one 7+ : 1*gray* in *Sordaria*. Their finding of no genuine wider ratio octads in *Ascobolus* does not therefore conflict with the finding here of many such octads.

## 4. DISCUSSION

The regular occurrence of genuine wider ratio octads, such as 8+ : 0*w*s and 7+ : 1*w*s, in the present crosses is evidence that hybrid-DNA can form in the same gene, at the same site of mutation, in both pairs of non-sister chromatids of a single bivalent. This is contrary to the prediction of von Wettstein's model (1971) of the synaptinemal complex, in which only two of the four chromatids are intimately paired at any one point. It is, however, consistent with other models of the synaptinemal complex (e.g. Henderson, 1971) which predict intimate pairing of both pairs of non-sister chromatids along the length of the synaptinemal complex. The double, side-by-side zipper arrangement in the Henderson (1971, see fig. 7) model and other similar models, permits, however, only two-strand double crossovers, implying complete negative chromatid interference, probably coupled with strong positive chromosome interference over very short distances. Published data on chromatid interference (e.g. Bole-Gowda, Perkins & Strickland, 1962) show no, or only weak, chromatid interference, with regular occurrence of three- and four-strand double crossovers. Even von Wettstein's model implies strong negative chromatid interference over very short distances.

The present evidence and previous chromatid interference data suggest that: all four chromatids can be paired, two by two, at a single site in the synaptinemal complex, and that partners in the non-sister pairing along the length of the synaptinemal complex change readily, with no strong negative chromatid interference.

In the corresponding-site interference studies, one anomalous feature was the difference in coincidence values from 7+ : 1*w* data (5·1 from low conversion crosses, 1·5 from high) and 8+ : 0*w* data (2·6 from low conversion crosses, 0·5 from high). The difference between the low conversion cross values is not significant ($\chi^2 = 0{\cdot}92$, $P = 0{\cdot}3$–$0{\cdot}5$) but it is for high conversion crosses ($\chi^2 = 12{\cdot}4, P < 0{\cdot}01$). If, as expected, correction of mis-paired bases is independent in both pairs of non-sister chromatids, this difference is unexpected.

Coincidence values were 3–5 times higher in low conversion crosses than in high conversion ones for both 7+ : 1*w* and 8+ : 0*w* data. Although no reason is known for this difference, it is not anomalous. In spite of such unexplained features of the data, the coincidence values showed that corresponding-site interference is weak rather than strong, and is certainly not completely positive or completely negative.

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